Delving deeper: metabolic processes in the metalimnion of stratified lakes

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Abstract

Many lakes exhibit seasonal stratification with strong thermal and chemical gradients. An expansion of depth-integrated monitoring programs has provided insight into the importance of organic carbon processing that occurs below the upper mixed layer. However, the chemical and physical drivers of metabolism and metabolic coupling in the metalimnion remain unresolved. Sharp gradients in key resources such as light and temperature co-occur in the metalimnion with dynamic physical conditions that influence metabolic processes directly and simultaneously hamper the accurate tracing of biological activity. We evaluated the drivers of metalimnetic metabolism and its associated uncertainty across ten stratified lakes in Europe and North America. We hypothesized that the metalimnion would contribute highly to whole-lake functioning in clear oligotrophic lakes, and that rates would be highly variable in unstable polymictic lakes. Depth-integrated rates of gross primary production (GPP) and ecosystem respiration (ER) were modelled from diel dissolved oxygen curves using a Bayesian approach. Metabolic estimates were more uncertain below the epilimnion, but uncertainty was not consistently related to lake morphology or mixing regime. Metalimnetic rates exhibited high day-to-day variability in all trophic states, with metalimnetic contribution to daily whole-lake GPP and ER ranging from 0 to 87% and <1 to 92%, respectively. Nonetheless, the metalimnion of oligotrophic lakes contributed highly to whole-lake metabolism, driven by a collinear combination of light, phosphorous concentration and metalimnetic volume. Further, higher background respiration was observed in the metalimnion of eutrophic lakes. We show that a single-sensor approach does not necessarily reflect whole-ecosystem carbon dynamics in stratified lakes.

Introduction

Globally, many lakes exhibit thermal stratification for a substantial portion of the year, developing marked vertical gradients in physical and chemical properties (Boehrer and Schultze 2008). These gradients influence biological communities and biogeochemical processes such organic and inorganic matter cycling (Coloso et al. 2008; Van de Bogert et al. 2012), altering energy flow through lake food webs (Wilkinson et al. 2014). Consequently, patterns of stratification affect the important contribution that inland waters make to global carbon fluxes (Cole et al. 2007; Coloso et al. 2008; Staehr et al. 2012b). Vertical patterns of ecosystem metabolism vary among chemically and morphologically diverse lakes (Obrador et al. 2014), but information on the drivers of metabolism in the metalimnion is currently limited. However, a recent expansion of high-frequency and depth-integrated monitoring of lakes provides an opportunity to extend our understanding of lake metabolism below the upper mixed layer (epilimnion) (Meinson et al. 2015; Obrador et al. 2014; Staehr et al. 2010).

Light availability is a key driver of gross primary production (GPP) below the upper mixed layer (Obrador et al. 2014; Sadro et al. 2011a; Staehr et al. 2012b). Light availability in the metalimnion is determined by two physical factors; the thickness of the epilimnion (Z_{mix}) and light attenuation through the water column (K_D). These characteristics vary distinctly among lakes with different chemical characteristics, potentially exerting strong control on metalimnetic processes. Nutrient availability in surface waters controls phytoplankton biomass, so that trophic state and light availability at depth are correlated. Planktonic communities below the epilimnion in eutrophic lakes may be shaded by high plankton densities in upper layers, while stratification reinforces nutrient limitation and relative clarity of surface waters of oligotrophic lakes (Obrador et al. 2014). Nutrient availability may additionally induce induces physiological responses in phytoplankton (e.g. cell size and stoichiometry) that affect their activity rates (Finkel et al. 2010). The chemical influences on GPP propagate to other ecosystem functions. Metabolic coupling between GPP and

ecosystem respiration (ER) is pronounced under oligotrophic conditions where heterotrophs are substrate-limited and depend on labile photosynthetic exudates (Sadro et al. 2014; Sadro et al. 2011b; Solomon et al. 2013).

In addition to distinct variation among lakes, day-to-day variation in ecosystem metabolism within lakes is substantial (Solomon et al. 2013). The variability differs in magnitude among lakes, and may be related to physical processes determined by lake morphology and mixing regime (Solomon et al. 2013). Periods of stable stratification may facilitate the development of metalimnetic algal communities (Pannard et al. 2015), while unstable and short-term stratification patterns could alter nutrients and OM fluxes, affecting temporal and spatial patterns of metabolic activity. However, attributing biological activity at a specific place and time to physical processes in the metalimnion poses a considerable challenge (Coloso et al. 2011; Staehr et al. 2012a). Biological signals on diel timescales are dampened in the metalimnion, which is a zone characterized by sharp vertical gradients in physical conditions. Therefore, hydrologic processes caused by external forcing (e.g. internal waves and advection; Boegman et al. 2003; Sadro et al. 2011a) need only be minor to contribute substantial noise to diel patterns. Such physical processes alter the spatiotemporal footprint of sensors used to measure the free-water dissolved oxygen (DO) concentrations that are used to estimate metabolic rates (Odum 1956). Thus, physical processes in the metalimnion may simultaneously influence on metabolic processes biologically and affect our ability to accurately trace them with high-frequency measurements.

Understanding the chemical and physical drivers of metalimnetic metabolism and its uncertainty among a diverse lake ecosystems would allow for a more accurate classification of stratified lakes as carbon sources or sinks at the ecosystem scale. We investigated vertical patterns of lake metabolism, photosynthetic light-use efficiency, and the coupling relationship between ER and GPP using high-frequency and depth-specific data from ten lakes and reservoirs that ranged in nutrient concentration and thermal stratification patterns. We used a

modelling technique that accounted for uncertainty in the estimation of metabolic parameters (following e.g. Batt and Carpenter 2012; Cremona et al. 2014b; Grace et al. 2015; Hanson et al. 2008). This approach offers several advantages; overcoming sources of error present in earlier 'book keeping' methods (McNair et al. 2013), and allowing for the quantification of variation in diel DO that is not explained by the model. We assumed that unexplained variation in diel DO was predominantly attributable to process errors caused by lateral movements of water that are not incorporated in the depth-integrated framework.

We hypothesized that light availability, as described by the ratio of the mixing depth (Z_{mix}) to the photic depth (Z_{eu}; determined by K_D) (i.e. $Z_{mix} : Z_{eu}$), would control biological processes in the metalimnion (Obrador et al. 2014; Sadro et al. 2011a; Staehr et al. 2012b). The relative contribution of the metalimnion to whole-lake metabolism was expected to be greatest in clear, oligotrophic lakes (i.e. low Z_{mix}:Z_{eu}). We sought to identify the conditions where depth-integrated estimates are useful to accurately estimate whole-lake NEP. It was expected that polymictic lakes with a more dynamic thermal structure would exhibit higher temporal variation explained by physical processes. Finally we utilized the depth specific metabolic estimates to investigate vertical patterns in the coupling between GPP and ER. Here we expected a tighter relation in the photic zone, especially in oligotrophic lakes, and ER to be more reliant on recalcitrant OM under low light conditions (Solomon et al. 2013; Obrador et al. 2014).

Methods

Study sites

We modelled open-water lake metabolism from high-frequency, depth-specific measurements of DO and water temperature (T) in ten lakes and reservoirs across Europe and North America. The dataset included monomictic, dimictic, and polymictic lakes with a range of morphological characteristics and chemical composition (Table 2). Concentration of nutrients,

dissolved organic carbon (DOC) and chlorophyll *a* (chl *a*) were determined from samples taken on 1-11 days at each lake during the stratified period (see Supplemental Text 1 for sampling and analytical methods). In addition, Chl-*a* concentration was estimated from high-frequency *in-situ* measurement using a fluorometer at some sites. Data from each lake represented a single year, for a period ranging from several weeks to many months. Only days when the water-column was thermally stratified (as described below) were included in analyses (17 to 69 days per lake).

The profiling systems or sensor chains recorded high-frequency measurements (10-60 min intervals) at 3 to 35 layers in each lake, with at least one measurement point in each depth zone (i.e. epi-, meta- and hypolimnion; Fig. 1; Table S1). We assumed that each measurement represented a horizontal layer of water centred on the measurement depth and having a thickness equal to the vertical resolution of measurements. Therefore, the deepest point of the profile was the depth of the deepest measurement plus half the vertical resolution of that layer. Meteorological data including photosynthetically active radiation (PAR), wind speed and barometric pressure were recorded at the same frequency as sub-surface measurements. Radiation measurements recorded as irradiance (E; W m^{-2}) were converted to photon flux in the 400-700 nm range (PAR; µmol m^{-2} s^{-1}) as follows (Kirk 1994; McCree 1981):

$$PAR = E \times 4.6 \times 0.45. \qquad (1)$$

Vertical stratification and underwater light regime

High vertical resolution (0.1 m) temperature profiles were modelled from available T data to determine if a lake was stratified on a given day and to delineate the metalimnion. T at each depth *z* was modelled as (Rimmer et al. 2005):

$$T(z) = T_h + (T_e - T_h)\left(\frac{1}{1+(\alpha \times z)^n}\right)^{1-\left(\frac{1}{n}\right)}, \qquad (2)$$

where T_e and T_h are the maximum temperature in the epilimnion and hypolimnion, respectively, and α and *n* are model-estimated constants. Temperature curves were fitted in a Bayesian framework with JAGS (Plummer 2003) using normally distributed, maximum-entropy priors. The metalimnion extent (i.e. Z_{mix} to the top of the hypolimnion) was defined using water density (ρ), calculated as:

$$\rho = 1 - 6.63 \times 10^{-6}(T - 4)^2. \quad (3)$$

The gradient in density between adjacent points that defined Z_{mix} was selected by visual inspection (Fig. S1) for each lake individually, and ranged between 0.03 and 0.18 kg m^{-3} m^{-1} (Read et al. 2011). The bottom of the metalimnion was similarly the deepest point with that density gradient. The sensitivity of metalimnetic volume and depth-zone specific metabolic rates to this gradient was examined at a subset of sites (Lake Ontario, Vedsted and Müggle; see Supplemental Text 2 and Table S2). The thermocline was defined between the points with the greatest density gradient (Read et al. 2011). Mean daily Z_{mix} and the bottom of the metalimnion were calculated as the mean values from all profiles on each day, and each layer was assigned to one depth zone (i.e. epi-, meta- or hypolimnion) for each day.

Water-column light attenuation (K_D) at each site was calculated from either measurements of underwater PAR at multiple depths, Secchi depth or other existing site-specific relationships (Table S1). Estimates of K_D were made from daily to biweekly intervals and were linearly interpolated between measurement days when necessary. Where underwater PAR measurements were available, K_D was estimated as the slope of the linear regression between log(PAR) and depth *z*. A mean daily K_D was calculated from the K_D of each profile during daylight (PAR_0 > 5 µmol m^{-2} s^{-1}) that had a linear regression fit with r^2 > 0.80 (Obrador et al. 2014; Staehr et al. 2012b). Where Secchi depth was available, K_D was calculated as 1.7/Secchi depth (Poole and Atkins 1929). The mean daily photic depth (Z_{eu}) was calculated as 4.6/K_D. Following the determination of daily K_D at each site, PAR for each

time interval and depth z (PAR_z) was calculated from incident PAR (PAR_0) using the Beer-Lambert law:

$$PAR_z = PAR_0 \times e^{-K_D \times z}. \quad (4)$$

Model of depth-dependent metabolism

Gaps in the data series of up to 1 h were linearly interpolated before we applied a 4-h simple moving average smoothing to DO, PAR and wind speed measurements (following Giling et al. 2016; Obrador et al. 2014). To estimate mean rates of ecosystem metabolism at a daily scale, we used a Bayesian model with non-linear sub-models for GPP and ER (Giling et al. 2016). Changes in DO concentration between successive measurement times t for each depth were partitioned into the contribution by biotic and physical processes using a depth-specific diel oxygen model (Staehr et al. 2012b):

$$DO(t+1) = DO(t) + NEP(t) - D_s(t) - D_v(t) + D_z(t)\,; \quad (5)$$

where NEP is the rate of net ecosystem production (mg O_2 L^{-1} h^{-1}), D_s is air-water exchange, D_v is diffusive vertical exchange between adjacent depths and D_z is metalimnetic exchange due to mixed-layer deepening. Atmospheric exchange (D_s) was applied only to layers in the epilimnion, and calculated as:

$$D_s(t) = K_s(t)\frac{DO_{mod}(t) - DO_{sat}(t)}{Z_{mix}(t)}. \quad (6)$$

The gas transfer velocity (K_s) was calculated at a Schmidt number of 600 (K_{600}) from wind speed standardized to 10 m height (U_{10}) according to Cole and Caraco (1998). In Equation 6, DO_{mod} is the modelled DO concentration following Song et al. (2016) and DO_{sat} is DO concentration at atmospheric equilibrium (calculated from temperature and barometric pressure). Vertical exchange between each depth and the adjacent depths due to turbulent diffusivity (D_v) was estimated using the Brunt-Väisälä buoyancy frequency (N^2 [s^{-2}], a measure of local stability; Boehrer and Schultze 2010) to calculate the vertical eddy

diffusivity coefficient (K_v) according to Hondzo and Stefan (1993). Lake Ontario was an exception due to its surface area exceeding the applicable range of the Hondzo and Stefan (1993) model. The metalimnetic K_v for Ontario was set as 6×10^{-6} m^2 s^{-1} based on measurements and theoretical work from Lakes Ontario and Erie (Bouffard et al. 2014; Sweers 1970). Epilimnetic and hypolimnetic K_v were expected to be higher than the metalimnetic estimate, so were set at Lake Ontario using a factor determined from the average relative difference between depth zones in the other nine lakes. The influence of K_v on metabolic rates at Ontario and two other lakes (Vedsted and Müggel) was assessed with a sensitivity analysis (for details see Supplemental Text 2 and Table S2). The flux D_z was calculated as proportional to the Z_{mix} deepening rate ($\Delta Z_{mix}/\Delta t$) and was applied to measurement points within the metalimnion and 1 m above or below (Obrador et al. 2014). At some sites, we set a threshold limiting the deepening rate to a maximum of 5 m h^{-1} to prevent short-term surface-water microstratification inaccurately affecting D_z in the metalimnion. For a full description of the model, see Staehr et al. (2012b).

Sub-models for the production and respiration components of *NEP* in Equation 5 were parameterized according to Grace et al. (2015). At each depth, GPP was modelled as a saturating function of PAR_z, while respiration was non-linearly dependent on T for each timestep t:

$$NEP(t) = A \times PAR(t)^p - R_{\bar{T}}(\theta^{(T(t)-\bar{T})}) ; \qquad (7)$$

where the first component describes GPP (mg O_2 L^{-1} h^{-1}), and A is a constant indicating production per unit light (i.e. photosynthetic efficiency), *PAR* is the depth-specific PAR (PAR_z), and p is an estimated exponent that represents the ability of producers to use light and accounts for saturating photosynthesis (when $p < 1$). The second component indicates DO consumption by ER (mg O_2 L^{-1} h^{-1}); $R_{\bar{T}}$ is the respiration rate at $\bar{T}$, Θ describes the temperature dependence of respiration (set to 1.072, corresponding to Q_{10} of 2; Wilcock et al. 1998), T is the water temperature at each timestep and $\bar{T}$ is the mean daily temperature in that

layer. Respiration was estimated at $\bar{T}$ and not standardized because we were interested in vertical patterns among the lakes at *in situ* conditions. Modelled rates were standardized to a temperature of 20°C (GPP_{20} and ER_{20}; for results see Fig. S2) only for analysis of coupling of ER_{20} to GPP_{20} (Holtgrieve et al. 2010; Solomon et al. 2013). We estimated the parameters *A*, *p* and $R_{\bar{T}}$ in Equation 7 for each depth with a Bayesian approach in JAGS (Plummer 2003) using R code (R Development Core Team 2014) modified from Grace *et al.* (2015) following the rationale of Song et al. (2016). Prior distributions for the estimated parameters were uniformly distributed within known physical constraints as described by Grace *et al.* (2015). Mean daily metabolic estimates (and their propagated uncertainty) for each depth layer were calculated from estimated parameters as:

$$GPP = \sum_{t=1}^{measurements} A \times PAR(t)^{p} \quad (8)$$

$$ER = 86400 \times \frac{R_{\bar{T}}}{\Delta t} \quad (9)$$

where *GPP* is daily gross primary production (mg O_2 L^{-1} d^{-1}), *ER* is daily ecosystem respiration at daily average temperature (mg O_2 L^{-1} d^{-1}) and 86400 converts from seconds to days. Convergence and stationarity of Markov Chain Monte Carlo (MCMC) values were assessed with the Gelman-Rubin convergence statistic $\hat{R}$ (Brooks and Gelman 1997). Models with $\hat{R} > 1.1$ (indicating unconverged chains) as well as poor fitting models ($r^2 < 0.40$) were excluded from further analyses. Excluding poor fitting models did not substantially alter vertical patterns of ecosystem metabolism or their uncertainty (Fig. S3). Example model fits for each lake are available in the supplementary material (Figs S7-S16) and example code for estimating depth-integrated metabolic rates is available online (github.com/dgiling).

Aggregating layer-specific metabolic rates

Mean daily rates in each depth zone (i.e. epi-, meta- and hypolimnion) were calculated by aggregating the appropriate daily estimates layer from each layer *i*. The standard deviation of

the aggregated depth zone rate (σ_{zone}) was propagated from the modelled uncertainties in layer *i* as follows:

$$\sigma_{zone} = \sqrt{\sum_i^n \sigma_i^2}\,, \qquad (10)$$

where *i* to *n* are the layers belonging to that depth zone. The aggregated depth-zone estimates were used for further analysis of metabolic rates. Due to high day-to-day variability, only days when there was at least one adequate metabolic model fit (i.e. $\hat{R} < 1.1$ and $r^2 > 0.40$) from layers in each depth zone (epi-, meta- and hypolimnion when present) were considered to calculate the relative contribution of the metalimnion to whole-lake metabolism (total of 178 days; Table 2).

Whole-lake metabolism estimates

Whole-lake volume-weighted estimates (WLVW; Sadro et al. 2011a) were calculated by multiplying the daily depth-zone volumetric rates (g O_2 m^{-3} d^{-1}) by the total volume (m^3) of each depth zone before summing the three zones. Metalimnetic volume was calculated using surface areas from hypsographic data and mean daily thickness from high-resolution temperature profiles. Whole-lake areal estimates were then obtained by dividing the WLVW estimate by the lake surface area (m^2). Hypolimnetic volume and thus metabolic contribution will be underestimated in the small number of lakes that were considerably deeper than the available profile measurements (e.g. Lake Ontario; Table 2). The proportional contribution of the metalimnion to whole-lake metabolism was calculated as the metalimnetic volumetric rate divided by the WLVW estimate (termed '$Prop_{metab}$'). We also assessed whether the metalimnion disproportionately contributed metabolic activity for its size (volume) for each day in each lake. We did this by calculating the ratio between the proportion of WLVW metabolism attributable to the metalimnetic ($Prop_{metab}$) and the metalimnetic contribution to

whole-lake volume (termed '$Prop_{vol}$'). Thus, the metalimnion contributed more to the whole lake metabolically than it did volumetrically when the ratio $Prop_{metab}$:$Prop_{vol}$ was >1.

We assessed how the traditional metabolism estimates based on single sensors in the epilimnion compared to depth-integrated estimates. 'Single-sensor estimates' were calculated by taking the sensor placed at 1 m depth, the most widespread DO sensor deployment depth (Solomon et al. 2013), and calculating whole-lake areal metabolism as above assuming that this rate was constant over depth. The shallowest available probe was used when no sensor was available at 1 m depth.

The 'background respiration' of OM not recently fixed can be inferred from the intercept of the relationship between daily ER_{20} and daily GPP_{20}, i.e. ER_{20} when $GPP_{20} = 0$ (del Giorgio and Williams 2005; Solomon et al. 2013). The slope of the relationship between daily ER_{20} and GPP_{20} describes the metabolic coupling, where a slope of 1 indicates a unit increase in ER_{20} for each unit increase in GPP_{20}. The coefficient of determination (r^2) indicates the strength of the coupling (Obrador et al. 2014). We used estimates from layers where there were >5 days with successful fits to estimate coupling regression parameters. Models were excluded where the layer was nearly always dark (so that GPP_{20} was zero or mean < 0.01 mg O_2 L^{-1} d^{-1}) because the slope was either vertical (and therefore undefined) or highly outlying (20-40 times the mean slope) and not conceptually meaningful. Coupling estimates for depth zone were calculated as the mean of all intercept and slope estimates from layers belonging to that depth zone.

Statistical analyses

Comparisons among depth zones, correlations with ancillary variables, and ER_{20}-GPP_{20} regressions were analyzed by fitting linear models or linear mixed models (LMMs) in R (R Development Core Team 2014). The LMMs included a random effect for site and layer and AR1 autocorrelation structure (nested within site) to account for repeated daily measurements

where appropriate. We compared a range of autocorrelation structures (including AR2 and AR3) with Akaike Information Criterion (AIC) values and found AR1 provided equivalent or better support for the models. Reported r^2 values refer to the variation explained by the fixed effects only. Variables were log-transformed when necessary. Principal components analysis (PCA) was used to reduce the number of collinear explanatory variables, and the resulting components were used as explanatory variables in LMMs to assess potential drivers of metalimnetic contribution.

Results

Uncertainty in model estimates and contribution of physical processes

The depth-integrated metabolic model provided a better description of diurnal changes in DO in the epilimnion (74% of models converged with adequate fit) than in the metalimnion (43%) or hypolimnion (32%; Fig. 2a). Furthermore, modelled metabolic estimates were on average more precise in the epilimnion (mean coefficient of variation [CV] for GPP and ER estimates = 0.14 and 0.23, respectively) than in the metalimnion (mean GPP and ER CV = 0.25 and 0.33, respectively) or hypolimnion (mean GPP and ER CV = 0.35 and 0.51, respectively) (Fig. 2b, c). There was high variability in model fit (R^2) and estimate certainty (CV) among days and depth zones (Fig. 2). In the metalimnion, R^2 was unrelated to water-column stability (assessed with the buoyancy frequency N^2; $F_{1,470} = 1.99$, $p = 0.159$; Fig. S4a). Accordingly, N^2 in successful models did not differ from N^2 in poor-fitting models (paired t-test, t = -0.004, $p = 0.997$; Fig. S4b). Variation in mean R^2 across all lakes and depth zones was also not explained by measurement frequency ($F_{2,25} = 0.445$, $p = 0.646$), the vertical resolution of measurements ($F_{1,26} = 0.000$, $p = 0.999$), Z_{max} ($F_{1,26} = 2.11$, $p = 0.159$) or surface area ($F_{1,26} = 0.441$, $p = 0.513$; Fig. S5). CV for GPP and ER was also unaffected or only marginally affected by measurement frequency ($F_{2,24} = 1.71$, $p = 0.202$ and $F_{2,25} = 3.377$, $p = 0.050$) and the vertical resolution of measurements ($F_{1,26} = 0.105$, $p = 0.749$ and $F_{1,26} = 0.167$, $p = 0.898$).

The physical fluxes of DO (i.e. D_s, D_v and D_z) contributed a substantial proportion (32 ± 24% across all estimates) of the total DO fluxes (i.e. sum of absolute NEP, D_s, D_v and D_z) (Table S3). In the epilimnion, a mean of 45% of DO changes were attributable to the diffusive components, mostly atmospheric exchange (D_s) *with a small contribution from* D_v and D_z. Transfer due to mixed layer deepening (D_z) contributed strongly to the metalimnion (mean 29% attributable to diffusive fluxes), and both D_z and D_v estimates were important in the hypolimnion (mean 16%; Table S3). The average magnitude of physical fluxes was unrelated to mean model R^2 ($F_{1,8} = 1.216$, $p = 0.302$) and CV of GPP and ER estimates among the ten lakes ($F_{1,8} = 3.499$, $p = 0.098$ and $F_{1,8} = 2.727$, $p = 0.173$). Further, we did not detect that the physical processes were not strongly affected by lake morphology. In the metalimnion, log-transformed lake area did not affect the balance between Dz, Dv and NEP with ($F_{1,8} = 1.218$, $p = 0.301$) or without ($F_{1,7} = 1.892$, $p = 0.211$) outlying Lake Ontario (Fig. S5). Sensitivity analysis demonstrated that the value of K_v did not strongly affect metabolic estimates, except for rates in the metalimnion and hypolimnion of polymictic Lake Müggle (Supplemental Text 2 and Table S2).

Metabolic estimates were characterized by high day-to-day variability in some lakes and layers (Fig. 1). For contiguous days with adequate model fits among the ten lakes, between 8-52%, 0-78% and 11-100% of epi-, meta- and hypolimnetic GPP estimates were within 2 standard deviations (SD) of the estimate from the previous day. Similarly, between 13-53%, 0-88% and 11-96% of epi-, meta- and hypolimnetic ER estimates were within 2 SD of the previous days' estimate. In the metalimnion, this did not appear to be strongly driven by PAR; the day-to-day shift in PAR_z did not consistently differ between consecutive days that had similar or disparate metabolic estimates. The proportion of days with estimates similar to the previous day was also not related to lake area (Fig. S5). Day-to-day variability in metalimnetic thickness as a proportion of Z_{max} was slightly higher in polymictic (SD in relative thickness = 0.08 ± 0.02, $n = 4$ lakes) than in mono-/dimictic lakes (SD = 0.04 ± 0.02,

381 n = 6 lakes, $F_{1,8}$ = 6.33, p = 0.04). However, metalimnetic local water-column stability did not
382 differ among mixing regimes ($F_{1,8}$ = 3.26, p = 0.109). The variability daily estimates of GPP
383 and ER in the metalimnion (Table S3) was not related to lake mixing regime ($F_{2,7}$ = 0.753, p =
384 0.505 and $F_{2,7}$ = 0.044, p = 0.957) or trophic status ($F_{2,7}$ = 1.337, p = 0.223 and $F_{2,7}$ = 0.178, p
385 = 0.841). We assessed whether process errors (e.g. lateral water movements) were responsible
386 for high day-to-day variability by testing whether the SD among daily estimates differed when
387 depth-zone means were aggregated from converged models with good (R^2 > 0.4) or poor fits
388 (R^2 < 0.4). We found was no difference in propagated SD for metalimnetic GPP (paired t-test;
389 t_9 = 1.42, p = 0.190) or ER (t_9 = 0.772, p = 0.460).
390
391 *Depth-specific metabolic rates and photosynthetic efficiency*
392 Vertical patterns of metabolism varied distinctly among the lakes, with mean GPP ranging
393 from 0.00 to 5.98 mg O_2 L^{-1} d^{-1} and ER from 0.00 to 3.74 mg O_2 L^{-1} d^{-1} across all depth zones
394 (Fig. 1; Table S3). Surface layers were most often autotrophic, whilst balanced to net
395 heterotrophic conditions were prevalent in the metalimnion. Mean NEP was < 0 mg O_2 L^{-1} d^{-1}
396 for 60% of the daily metalimnetic estimates across all the lakes and layers. Mean daily GPP in
397 the epilimnion was positively correlated with mean epilimnetic TP concentration ($F_{1,7}$ = 8.75,
398 p = 0.021, r^2 = 0.56), as was epilimnetic ER ($F_{1,7}$ = 10.59, p = 0.014, r^2 = 0.60). In the
399 metalimnion, mean GPP and ER were not linearly correlated to epilimnetic TP concentration
400 ($F_{1,7}$ = 2.04, p = 0.196, r^2 = 0.22 and $F_{1,7}$ = 0.10, p = 0.404, r^2 = 0.10, respectively; Fig. 3a,b).
401 We found evidence of photosynthetic activity down to *ca.* 0.1% of surface light.
402 Increasing GPP with depth in some lakes was due to higher daily photosynthetic efficiency
403 (i.e. parameter A from Equation 7; GPP [mg O_2 L^{-1} d^{-1}] / PAR [µmol m^{-2} sec^{-1}]) in low-light
404 conditions ($F_{1,792}$ = 105.17, p < 0.001; Fig. S6). Correspondingly, photosynthetic efficiency
405 varied by depth ($F_{2,809}$ = 58.55, p < 0.001), and was lower in the epilimnion than in the
406 metalimnion or hypolimnion (p < 0.01 in post-hoc pairwise comparisons), which did not

differ (p = 0.905). Photosynthetic efficiency was reduced in low nutrient conditions ($F_{2,23}$ = 25.72, p < 0.001). Efficiency was significantly lower in oligotrophic lakes than in mesotrophic lakes (post-hoc comparison; z = -4.54, p < 0.001), which were lower again than eutrophic lakes (z = -3.17, p = 0.004).

Metalimnetic contribution to whole-lake metabolism

Across all sites, the proportional contribution of the metalimnion to WLVW metabolic estimates (i.e. $Prop_{metab}$) varied between 0 and 87% for daily GPP and between <1 and 92% for daily ER (Fig. 4a,b). The metalimnetic $Prop_{metab}$ was negatively correlated to epilimnetic TP concentration for GPP ($F_{1,7}$ = 6.09, p = 0.042, r^2 = 0.47) and for ER ($F_{1,7}$ = 8.69, p = 0.021, r^2 = 0.55), but these relationships were driven by Lake Castle (Fig. 3c,d). The proportion of whole-lake volume made up of the metalimnion (i.e. $Prop_{vol}$) was between 3 and 60% (mean 32%). The metalimnetic $Prop_{metab}$ was positively correlated with metalimnetic $Prop_{vol}$ for both GPP (slope = 0.88 ± 0.12, $F_{1,175}$ = 58.46, p < 0.001, r^2 = 0.25) and ER (slope = 0.94 ± 0.14, $F_{1,175}$ = 43.74, p < 0.001, r^2 = 0.20). The metalimnetic $Prop_{metab}$:$Prop_{vol}$ was a mean 0.95 ± 0.67 SD for GPP and 1.06 ± 0.87 SD for ER. The ratio $Prop_{metab}$:$Prop_{vol}$ showed that the metalimnion disproportionately contributed metabolic activity for its size (i.e. had high $Prop_{metab}$:$Prop_{vol}$) in oligotrophic lakes (Fig. 3e,f). This was evidenced by a negative relationship between metalimnetic $Prop_{metab}$:$Prop_{vol}$ and mean epilimnetic TP concentration for GPP ($F_{1,7}$ = 15.31, p = 0.006, r^2 = 0.68; Fig. 3e) and ER ($F_{1,7}$ = 12.46, p = 0.010, r^2 = 0.64; Fig. 3f).

We observed a negative linear correlation between metalimnetic $Prop_{metab}$ and log-transformed Z_{mix}:Z_{eu} for GPP ($F_{1,166}$ = 12.39, p < 0.001; Fig. 4a). However, this trend was not robust to the exclusion of Lake Castle ($F_{1,151}$ = 2.47, p = 0.118). A similar negative relationship was observed for metalimnetic ER $Prop_{metab}$ (Fig. 4b), with ($F_{1,166}$ = 18.58, p < 0.001) or without ($F_{1,151}$ = 12.10, p < 0.001) Lake Castle. Comparable trends were observed

433 for the effect of log-transformed Z_{mix}:Z_{eu} on $Prop_{metab}$:$Prop_{vol}$, with a negative relationship for
434 GPP ($F_{1,166}$ = 15.22, p < 0.001; Fig. 4c), which was again not significant when Castle Lake
435 was removed from the analysis ($F_{1,151}$ = 2.08, p = 0.151). In contrast, the negative effect of
436 log-transformed Z_{mix}:Z_{eu} on $Prop_{metab}$:$Prop_{vol}$ for ER ($F_{1,166}$ = 12.46, p < 0.001; Fig. 4d) was
437 robust to the exclusion of Castle Lake ($F_{1,151}$ = 8.31, p = 0.005). Metalimnetic $Prop_{vol}$ tended
438 to be high when Z_{mix} was shallow, meaning that log Z_{mix}:Z_{eu} and $Prop_{vol}$ were negatively
439 correlated ($F_{1,166}$ = 13.20, p < 0.001, r^2 = 0.11). Hence, we used PCA to examine the collinear
440 drivers of metalimnetic $Prop_{metab}$. Principal components (PCs) were derived from four
441 variables representing abiotic resource availability and physical conditions (PAR_z,
442 temperature, $Prop_{vol}$ and N^2). The PC explaining the majority of variation (PC1) did not
443 reduce the collinearity, as it was characterized by lower temperature, $Prop_{vol}$ and N^2 (Table 3).
444 Metalimnetic $Prop_{metab}$ for GPP and ER was negatively correlated to PC1 in linear mixed
445 models (Table 3), suggesting a combined importance of these factors. A second component
446 (PC2) was characterized mainly by low light and high local stability N^2 in the metalimnion,
447 and was negatively correlated with $Prop_{metab}$ for GPP and ER (Table 3).
448
449 *Depth-specific metabolic coupling*
450 Coupling between ER_{20} and GPP_{20} varied among the lakes and depth zones. Background
451 respiration (intercept) ranged from -0.16 to 2.70 mg O_2 L^{-1} d^{-1}, mean coupling slope ranged
452 from -0.07 to 1.26 and mean r^2 varied between 0.05 and 0.94 (Table S4). There was an
453 interaction between the effect of depth zone and epilimnetic TP concentration on mean
454 background respiration ($F_{2,17}$ = 7.19, p = 0.005). Mean epilimnetic TP did not affect mean
455 background respiration in the epilimnion ($F_{1,7}$ = 4.64, p = 0.068; Fig. 5a), but there was a
456 positive linear correlation in both the metalimnion ($F_{1,7}$ = 26.21, p = 0.002; Fig. 5b) and
457 hypolimnion ($F_{1,7}$ = 19.19, p = 0.012; Fig. 5c). The positive relationship in the hypolimnion
458 was driven predominantly by Lake Müggel (Fig. 5c). Across all TP levels, background

459 respiration did not differ among depth zones ($F_{2,22} = 1.13$, $p = 0.340$). There was no
460 interaction between the effect of epilimnetic TP and depth zone on the coupling slope ($F_{2,17} =$
461 1.20, $p = 0.324$), and no significant difference among depth zones ($F_{2,17} = 0.44$, $p = 0.653$).
462 Across all depth zones, the coupling slope deceased with increasing epilimnetic TP
463 concentration ($F_{1,17} = 6.48$, $p = 0.021$), but this relationship was not evident in any one depth
464 zone individually ($p > 0.117$; Fig. 5d-f). For the strength of the coupling relationship (r^2) there
465 was also no interaction between the effect of depth zone and mean epilimnetic TP ($F_{2,17} =$
466 0.885, $p = 0.431$), and no effect of depth zone ($F_{2,17} = 0.023$, $p = 0.977$). The strength of the
467 coupling relationship decreased with increasing TP concentration ($F_{1,17} = 7.97$, $p = 0.012$)
468 across all depth zones combined (Fig. 5g-i).
469
470 *Effect of depth-integration on whole-lake areal NEP estimate*
471 We assessed how WLWV estimates of GPP, ER and NEP made from a single epilimnetic
472 sensor compared to the depth-integrated estimates. For mean GPP, the single-sensor approach
473 over-estimated whole-lake metabolism at 9 of 10 sites, but the average difference from
474 integrated estimates was variable (mean difference 3.17 ± 4.58 g O_2 m^{-1} d^{-1}; Fig. 6a). In
475 contrast, single-sensor estimates of ER did not consistently vary from integrated estimates
476 (mean difference -0.37 ± 3.40 g O_2 m^{-1} d^{-1}; Fig. 6b). Correspondingly, single-sensor estimates
477 of whole-lake NEP generally were slight overestimates, but there was considerable variation
478 both among lakes and among days within lakes (mean difference 3.55 ± 6.65 g O_2 m^{-1} d^{-1}; Fig.
479 6c).
480
481 **Discussion**
482 We found contrasting patterns in the vertical structure of metabolic rates among the stratified
483 lakes using a large depth-integrated and high frequency buoy dataset (Fig. 1). In clearer lakes,
484 mean daily rates of GPP and ER were weakly associated with depth (e.g. Lake Bure) or

peaked in the metalimnion (e.g. Lakes Ontario, Stechlin), as reported before (Sadro et al. 2011a). In contrast, metabolic rates of some mesotrophic and eutrophic lakes were strongly depth-dependent (e.g. Lake Castle; Fig. 1). In lakes with high vertical variation in rates, single-sensor estimates of whole-lake metabolism deviated considerably from depth-integrated estimates on some days (Fig. 6). Despite dissimilar vertical profiles, we found that the metalimnion can contribute substantially to daily whole-lake metabolism across a broad range of lakes, with the exception of highly eutrophic conditions (Figs. 3 & 4). However, the relative contribution of the metalimnion varied substantially among days regardless of lake stratification pattern, and metalimnetic $Prop_{metab}$ was only moderately explained by changes in light availability (as indicated by Z_{mix}:Z_{eu}). Nonetheless, the metalimnion of oligotrophic lakes, with more light available at depth, contributed more on average to lake metabolism than expected from their metalimnetic volume ($Prop_{metab}$:$Prop_{vol}$ > 1; Fig. 3e,f).

Importance of physical processes to model uncertainty and day-to-day variability

Our depth-integrated analysis extends current understanding of the role that physical processes play in obtaining accurate metabolic estimates (Coloso et al. 2008; Staehr et al. 2012b; Van de Bogert et al. 2007). Combined with previous sensitivity analyses (Obrador et al. 2014), we show that daily estimates of metabolic rates are robust to uncertainties in vertical diffusive fluxes (D_v) under many circumstances, but there were exceptions in the deeper layers of a polymictic lake (Table S2). This increased sensitivity was likely due to the higher contribution of D_v and D_z to DO fluxes in deeper layers and corresponding decrease in the diel signal of DO, water temperature and PAR (Figs S7-S16), as well as model fit (Fig. 2). We expected that high variation in patterns of stratification or low water-column stability would be associated with poor fitting models and uncertainty in parameter estimates. However, within the metalimnion we found no evidence that polymictic lakes, or days and layers with low stability were associated with poor model convergence or fit. Furthermore,

511 model fit and uncertainty were similar among lakes and unrelated to mixing regime (Fig. 2).
512 Hence, while the accuracy of K_v can influences metabolic results, it does not appear that the
513 magnitude or accuracy of estimated diffusive fluxes are important factors in our ability to
514 accurately describe diel variation in DO. This was supported by total diffusive fluxes
515 (including D_s) being high in the epilimnion where model fits were often very good.
516 There are a range of physical processes that were not accounted for by the free-water
517 framework utilized here, including horizontal advection, metalimnetic intrusions and internal
518 seiches caused by wind forcing (Solomon et al. 2013; Van de Bogert et al. 2007). These
519 process errors bring water masses influenced by heterogeneous chemical conditions and
520 biological communities (Solomon et al. 2013; Van de Bogert et al. 2012; Van de Bogert et al.
521 2007). For example, Sadro *et al.* (2011a) found that pelagic rates were commonly influenced
522 by littoral habitats due to advection and water-column stability. The patterns of model fits
523 found among depth zones suggested physical process errors were a considerable issue in the
524 meta- and hypolimnion. Because physical processes are a function of lake morphology, we
525 expected the degree of uncertainty to vary among lakes. However, we did not observe any
526 consistent trends in parameters of model fit or importance of diffusive fluxes across our
527 gradient of lake areas (Fig S6), suggesting process areas did not systematically bias our
528 interpretations. However, lake area is confounded by other variables in our dataset including
529 measurement frequency (Table S1).
530 Physical processes in addition to diffusion may have contributed to the high day-to-day
531 variability in the metabolic estimates because we found little evidence that variability in
532 metalimnetic rates was driven by biological responses (e.g. due to differences in PAR).
533 Conversely, high day-to-day variability is typical even of surface-water metabolic rates that
534 are estimated with higher certainty and better model fits (Solomon et al. 2013; current study).
535 Furthermore, selecting models with good or poor fit did not affect among-day variability or
536 vertical patterns in metabolism (Fig S3). This suggested either that R^2 was a poor predictor of

537 the importance of process errors, or that process errors did not contribute highly to among-day
538 variability. Quantifying the effect of physical processes on metabolic estimates using free-
539 water measurements remains a considerable challenge and priority for future research.
540 Ecological inferences made using free-water methods should remain coupled to this
541 consideration (Sadro et al. 2011a) and be interpreted in the context of model fit and
542 uncertainty (Cremona et al. 2014b).

543 Our results suggest we should remain cautious that patterns in metabolic activity are
544 attributable to biological activity occurring at a certain place and time, especially when
545 stratification periods are not prolonged. However, we employed a number of methods to assist
546 in providing useful information on metabolic processes in the deeper layers of stratified lakes.
547 These included analyzing weeks to months of data for each lake, and using methods such as
548 temporal smoothing and calculating stratification and diffusive fluxes on sub-daily timescales
549 (Coloso et al. 2011) was expected to. Further, where depth-integrated measures of chl-*a*
550 concentration were available (Table S1), vertical patterns in metabolism generally matched
551 algal biomass. In our study, the vertical resolution of measurements did not affect estimate
552 certainty, but maintaining a high resolution will increase the accuracy of temperature profiles
553 and stratification patterns. This is important for calculating the contribution of specific lake
554 habitats, because metalimnetic contribution was sensitive to its thickness (Supplemental Text
555 2 and Table S2). Vertical resolution will also assist in cases such as Lake Castle where the
556 development of hypolimnetic hypoxia (Fig. S9) could produce unrealistically sharp diffusive
557 gradients if too few sensors are used.

558

559 *Vertical patterns in efficiency of light utilization and metabolic coupling*

560 The increase in light utilization efficiency in deeper depth zones explained the higher GPP
561 rates in the metalimnion despite lower light availability. However, the mechanisms
562 responsible for this pattern may vary among lakes. Chl-*a* concentration available at multiple

depths from subset of lakes showed that increases in metalimnetic photosynthetic efficiency at Lakes Hampen, Vedsted, Castle (Obrador et al. 2014) and El Gergal (Table 2) were not caused by higher algal biomass in the metalimnion. This suggests that the phytoplankton communities at depth were physiologically acclimated to the low-light conditions or benefited from the potentially higher nutrient availability and lower temperatures that reduce metabolic costs. In other lakes, such as oligotrophic Lakes Abant and Stechlin, and mesotrophic Lake Bure, chl-*a* concentration peaked in the metalimnion. Deep chlorophyll maxima (DCM) are a common occurrence in many lakes (e.g. Brentrup et al. in press; Hamilton et al. 2010), and their development will have a strong impact on vertical patterns of metabolism and metabolic coupling. Photosynthetic efficiency may also reflect the physiology of the species comprising the phytoplankton community. Rates of GPP and ER above Z_{eu} were conspicuously low at El Gergal for a mesotrophic system, potentially attributable to the dominance by a large, inefficient and slow-growing cyanobacterium (*Aphanizomenon flos-aquae*; Moreno-Ostos et al. 2016).

Our estimates corroborate with previous findings that heterotrophic to balanced conditions generally prevail in the metalimnion (e.g. Sadro et al. 2011a), even when it was presumably well lit (Fig. 4e). This has been previously ascribed to ER being less depth-dependent than GPP (Coloso et al. 2008). However, vertical patterns in ER were similar to GPP at many sites, at both *in situ* temperature and when standardized to 20°C (Figs. 1 & S2). Days of autotrophy in the metalimnion of lakes from all trophic states and mixing regimes interspersed the predominant state of heterotrophy (Wilkinson et al. 2015). Despite high variability in GPP and ER rates among sites, metalimnetic NEP was relatively stable (Fig. 4e), with a mean of -0.08 ± 0.26 mg O_2 L^{-1} d^{-1} among lakes when excluding Lake Müggle, which had outstandingly high autotrophy in the metalimnion (Fig. 1). This relative stability was likely due to the metalimnetic ER_{20}-to-GPP_{20} coupling gradient being close to 1 at many sites (Fig. 5e). Substrate limitation of heterotrophs is hypothesized to drive strong metabolic

589 coupling (Sadro et al. 2011b; Solomon et al. 2013), and may be pronounced at depth because
590 the OM in deeper layers typically contains a greater proportion of recalcitrant molecules with
591 low biodegradability (Ostrom et al. 1998). A small number of mean coupling slope estimates
592 > 1 were observed in the meta- and hypolimnion (Fig. 5e, f), suggesting greater than unit
593 increases in ER for each unit increase in GPP. Priming of recalcitrant OM (Guenet et al.
594 2010) could produce such a pattern but evidence for this process in freshwater systems is
595 inconclusive (Catalán et al. 2015). As these high slope estimates all occurred at low GPP it is
596 more likely that these estimates were artificially inflated by statistically influential data points
597 with low GPP relative to ER.
598 Elevated background respiration in the meta- and hypolimnion of some mesotrophic and
599 eutrophic sites suggests respiration of OM that was not recently or locally produced (Solomon
600 et al. 2013). The metalimnion may be a zone with high degradation of particles that
601 accumulate near the thermocline through a number of co-occurring mechanisms (Staehr et al.
602 2012b). This includes recently-produced organic material sinking from the epilimnion,
603 combined with large pools of recalcitrant dissolved organic matter (DOM). Other particles
604 such as zooplankton carcasses may be highly available in the metalimnion because they have
605 higher residence times in stratified water columns and move slowly through the thermocline
606 (Kirillin et al. 2012). Particulate organic matter (POM) accumulating below the mixed layer
607 could also originate from resuspension of benthic material by internal seiches during
608 stratification (Weyhenmeyer 1996) or external stream- and groundwater inputs that do not
609 mix with the epilimnion. Higher nutrient availability below the epilimnion may also facilitate
610 greater decomposition rates. For mean heterotrophic conditions, there must be a net subsidy of
611 carbon to the metalimnion and hypolimnion. In addition to accumulating particles, diel
612 vertical migrations could play a role in linking surface and deeper waters, which has been
613 relatively unexplored compared to physical mechanisms. Daily rates of microbial background
614 respiration in the metalimnion could be spatially subsidized by zooplankton that migrate to

surface waters at night and return to excrete epilimnion-derived DOM and POM at depth (Watras et al. 2015). Furthermore, some motile autotrophs and mixotrophs vertically migrate to the surface to photosynthesize during the day (Salonen et al. 1984), and so respire carbon in deeper waters that was fixed in the surface waters.

Drivers of metalimnetic contribution to whole-lake metabolism

Contrary to expectations, light conditions in the metalimnion, as measured by the relation between mixing depth and photic zone (Z_{mix}:Z_{eu}), did not explain a large portion of the variation in metalimnetic $Prop_{metab}$ or $Prop_{metab}$:$Prop_{vol}$ alone (Fig. 4). Instead, our results suggested a collinear combination of physical and chemical variables, including light, nutrient concentration, temperature, water-column stability and metalimnetic volume drives metalimnetic $Prop_{metab}$ (Figs. 3 & 4, Table 3). We did not find a strong association between mixing regime and metalimnetic $Prop_{metab}$, but the contribution will naturally be more temporally dynamic in polymictic lakes that have interspersed periods of mixed water columns. Concentration of TP in the epilimnion was negatively correlated with metalimnetic $Prop_{metab}$:$Prop_{vol}$, likely due to stimulating epilimnetic production that shades metalimnetic organisms (e.g. Laas et al. 2012). However, trends for GPP were highly influenced by the most eutrophic lakes, so that additional sites are required to confirm these patterns. Despite the decreasing metalimnetic $Prop_{metab}$, absolute rates in the metalimnion increased slowly with TP concentration, until a threshold where the metalimnion was shaded (Castle Lake; Fig. 3a,b). These results imply that eutrophication of lake ecosystems may shift primary production from the metalimnion to the epilimnion, as observed for coastal systems (Lyngsgaard et al. 2014).

The variability in metalimnetic contribution to whole-lake metabolism was highly evident in the range of 0.5 to 1.0 Z_{mix}:Z_{eu} (Fig. 4). While interpolating K_D could introduce error, such variability was evident even at sites with sub-daily determination of K_D (e.g. Lake

641 Stechlin). The dynamic nature of metalimnetic volume on both daily and seasonal bases
642 (Coloso et al. 2011) is likely to affect its contribution to the depth-integrated areal rates
643 (Staehr et al. 2012b). Fluctuations in thermal structure represent not only shifts in light
644 availability, but also potentially important fluxes of OM, nutrients and biota among layers or
645 between sediments and the water column. These fluxes may partly account for the high
646 variability in metabolic estimates at polymictic Lake Müggel, where the longest run of
647 consecutive stratified days was seven. In addition, mixing caused by wind or rain might
648 stimulate metabolism by providing a nutrient or OM subsidy (Giling et al. 2016; Johengen et
649 al. 2008), or depress GPP by suspending OM (Sadro and Melack 2012; Tsai et al. 2008). A
650 key priority to further understand the chemical and physical drivers of variability in the
651 metabolism of stratified lakes is obtaining a greater vertical and temporal resolution of data on
652 nutrient concentration and OM composition (e.g. Watras et al. 2015; Wilkinson et al. 2014).
653 The multiple interacting factors that influence processes below the upper mixed layer
654 suggest that metabolic rates and metalimnetic contribution to whole-lake metabolism may be
655 sensitive to a range of human influences. Anthropogenic changes pervasively affect patterns
656 of thermal stratification and light attenuation in a multitude of ways (Adrian et al. 2009;
657 Carpenter et al. 2011; Tanentzap et al. 2008). For example, climate change may reinforce the
658 thermal stratification and gradually shift mixing regimes (Butcher et al. 2015; Kirillin 2010;
659 Kraemer et al. 2015; Livingstone 2008), while extreme events will disrupt stratification
660 (Jennings et al. 2012). Widespread brownification and cyanobacterial blooms will both shade
661 the water column and alter food-web dynamics (Cremona et al. 2014a; Paerl and Paul 2012;
662 Solomon et al. 2015). In some cases these shifts need only be minor to have a large impact on
663 vertical patterns of metabolism. In particular, biological processes in the metalimnion of lakes
664 with Z_{mix}:Z_{eu} close to unity may have little resistance to altered light attenuation or thermal
665 structure. These changes have implications for carbon efflux or storage at regional and even
666 global scales (Tranvik et al. 2009).

In conclusion, we found that the metalimnion can contribute substantially whole-lake metabolism in many lakes using a depth-integrated approach. However, high variability in rates and collinearity among predictors meant that generalisations about the widespread importance of the metalimnion to water-column processes could not be made with broad lake categories such as trophic status, except for hypereutrophic lakes. Single sensors placed in the epilimnion sensors retain value, especially for investigating the drivers of day-to-day variation in long term records due to high reliability of estimates and relative freedom from process errors. However, single-sensor estimates may not necessarily reflect whole-lake functioning; and deviate from systematically from depth-integrated estimates of GPP. A depth-integrated approach enables enhanced understanding of how physical and biogeochemical processes influence functioning of lake ecosystems as a whole.

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880 **Tables**

881 Table 1. Description of acronyms and terms

Parameter or acronym	Description	Unit
OM	Organic Matter	
GPP	Gross Primary Production at in situ temperature	mg O_2 L^{-1} d^{-1}
GPP_{20}	Gross Primary Production standardized to 20°C	mg O_2 L^{-1} d^{-1}
ER	Ecosystem Respiration at in situ temperature	mg O_2 L^{-1} d^{-1}
ER_{20}	Ecosystem Respiration standardized to 20°C	mg O_2 L^{-1} d^{-1}
Background respiration	Respiration of OM not recently or locally fixed; the intercept of the correlation between ER20 and GPP20	mg O_2 L^{-1} d^{-1}
NEP	Net Ecosystem Production (GPP-ER); describes whether the layer or lake is net autotrophic or net heterotrophic	mg O_2 L^{-1} d^{-1}
TP	Total Phosphorous	µg L^{-1}
TN	Total Nitrogen	µg L^{-1}
DO	µg L^{-1}	mg L^{-1}
Z_{mix}	Mixing depth; bottom of the epilimnion and top of the metalimnion	m
Z_{eu}	Photic depth; equal to depth with 1% of surface light	m
Z_{mix} : Z_{eu}	Ratio of mixing to photic depth. Describes light availability in the metalimnion	
T	Temperature	°C
DOC	Dissolved Organic Carbon	mg L^{-1}
Chl *a*	Chlorophyll-*a* concentration	µg L^{-1}
DCM	Deep Chlorophyll Maximum	
PAR_0	Incoming surface Photosynthetic Active Radiation	µmol m^{-2} s^{-1}
PAR_z	Photosynthetic Active Radiation at depth z	µmol m^{-2} s^{-1}
K_D	Light attenuation coefficient	m^{-1}
U_{10}	Wind speed at 10 m above surface of lake	m s^{-1}
D_s	Atmospheric	mg O_2 L^{-1} h^{-1}
D_v	Vertical exchange between each depth and the adjacent depths due to turbulent diffusivity	mg O_2 L^{-1} h^{-1}
N^2	Brunt-Väisälä buoyancy frequency	s^{-2}
K_v	Vertical eddy diffusivity coefficient	
D_z	Exchange due to mixed-layer deepening	mg O_2 L^{-1} h^{-1}
WLVW	Whole-lake volume-weighted estimate of metabolism (Sadro et al. 2011a)	g O_2 m^{-3} d^{-1}
$Prop_{metab}$	Proportion of WLWV metabolism occurring in a certain depth zone	
$Prop_{vol}$	Proportion of whole lake volume occurring in a certain depth zone	
$Prop_{metab}$: $Prop_{vol}$	Ratio of proportion metabolism to proportion volume; ratio is >1 when a depth zone contributes more metabolically than it does volumetrically	

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Table 2. Site information and ancillary data for the ten study lakes. Chemical and biological data are mean measurements from 1-11 samples among sites during the period of stratification at each lake (Supplemental Text 1). 'Epi.' denotes the epilimnion (metalimnion and hypolimnion measurements are presented in Table S1). The column '# days (total)' shows the total number of stratified days with metabolic estimates in each lake, with the number of days with at least one successful model fit in each depth zone (epi-, meta- and hypolimnion) in brackets.

Lake	Location	# days	Trophic status	Mixing regime	Max. depth (lake)	Max. depth (profile)	Mean Z_{mix}	Mean Z_{eu}	Mean meta. thickness	Epi. TP	Epi. TN	Epi. DOC	Epi. Chl *a*
					m	m	m	m	m	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}
Abant	Turkey	17 (2)	oligo.	mono.	18	12.75	5.6	11.9	2.6	14.0	1.5		1.5
Ontario	United States	69 (17)	oligo.	mono.	244	35	10.3	23.2	10.3	6.4			0.7
Stechlin	Germany	41 (32)	oligo.	di.	69.5	17.25	6.8	12.7	5.1	12.2	0.4	5.0	1.9
Rappbode pre-dam	Germany	48 (19)	meso.	di.	17	15.75	2.6	4.7	4.7	25.2	0.7	4.8	9.4
Bure	Denmark	58 (29)	meso.	poly.	11	9.5	4.3	7.5	3.3	22.9	0.7		5.5
Hampen	Denmark	56 (15)	meso.	poly.	13	9.5	4.4	6.3	3.1	23.6	0.6	3.0	8.7
El Gergal	Spain	21 (17)	meso.	mono.	37	19.75	5.3	6.5	6.1				24.1
Vedsted	Denmark	60 (14)	eu.	di.	11	9.5	3.5	4.0	3.8	27.3	0.5	4.9	41.4
Müggel	Germany	28 (17)	eu.	poly.	7.7	5.25	1.5	3.0	2.0	63.5	0.8	7.2	35.3
Castle	Denmark	32 (16)	hypereu.	poly.	9	8.5	4.4	2.3	2.6	94.1	1.6	3.4	46.3

890 Table 3. Results of principal components analysis (PCA). 'Linear mixed models' rows indicate the results of linear models using the components as
891 explanatory variables to explain metalimnetic contribution to whole-lake areal GPP and ER ($Prop_{metab}$). 'β' indicates the estimated slope of the
892 linear model.

Principal component	PC1	PC2
% variation explained	36	32
Axis rotation		
Metalimnetic $Prop_{vol}$	-0.52	-0.42
Mean metalimnetic log PAR_z	-0.26	-0.65
Mean metalimnetic layer temperature	-0.68	0.19
Mean metalimnetic buoyancy frequency (N^2)	-0.43	0.60
Linear mixed models		
$Prop_{metab}$ for GPP	β = -0.11 ± 0.01 p < 0.001, r^2 = 0.26	β = -0.02 ± 0.02 p = 0.200, r^2 = 0.08
$Prop_{metab}$ for ER	β = -0.07 ± 0.02 p < 0.001, r^2 = 0.09	β = -0.10 ± 0.02 p < 0.001, r^2 = 0.15

Figure captions

Figure 1. Mean (± SD among days) depth-specific volumetric estimates of daily GPP (open white points) and ER (closed grey points) of adequately fit models over stratified days at each lake. The grey rectangle indicates the mean extent of metalimnion and the dashed black line indicates the mean daily Z_{eu}.

Figure 2. Scatterplots of mean (± SD) (a) R^2, (b) coefficient of variation (CV) of GPP estimates, and (c) CV of ER estimates from all models across the study lakes and depth zones. White, grey and black points show epi-, meta- and hypolimnetic zones respectively. Value above the dashed horizontal line at $R^2 = 0.40$ in panel (a) were considered to have adequate model fit.

Figure 3. Scatterplots showing the correlation between mean (± SD) epilimnetic total phosphorus (TP) and metalimnetic GPP (left) and ER (right). Plots show mean (± SD) metalimnetic volumetric rates (a, b), mean relative contribution of the metalimnion to WLVW metabolic estimates (i.e. $Prop_{metab}$; c,d); and the ratio between $Prop_{metab}$ and the relative contribution of the metalimnetic volume to whole-lake volume ($Prop_{metab}$: $Prop_{vol}$; e,f). Solid black lines indicate significant linear relationships in linear mixed models. White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares/diamonds and triangles represent monomictic, dimictic and polymictic lakes, respectively.

Figure 4. Scatterplots showing the correlation between Z_{mix}:Z_{eu} and metalimnetic metabolism. The top row shows the proportional contribution of the metalimnion to WLVW metabolic estimates ($Prop_{metab}$) for (a) GPP and (b) ER. The middle row shows the effect of Z_{mix}:Z_{eu} on the ratio between $Prop_{metab}$ and the proportion contribution of the metalimnetic volume to

whole-lake volume ($Prop_{metab}$:$Prop_{vol}$) for (c) GPP and (d) ER. At values higher than the dashed horizontal line at $Prop_{metab}$:$Prop_{vol}$ = 1 the metalimnion contributes more to the whole lake metabolically than it does volumetrically. Panel (e) shows the volumetric rate of NEP as a function of mean daily Z_{mix}:Z_{eu}. Each point represents one day, with a point only for days with at least one appropriate model fit in each depth-zone shown for plots a-d. White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles represent monomictic, dimictic and polymictic lakes, respectively. In plots c and d some outlying high points at Lake Ontario were excluded to assist interpretation.

Figure 5. Scatterplots of the correlation between and nutrient concentration on ER_{20}-to-GPP_{20} coupling in the epi- (left), meta- (centre) and hypolimnion (right). The top row shows the effect of mean (±SD) epilimnetic TP on mean (±SD) background respiration (i.e. intercept of correlation; mg O_2 L^{-1} d^{-1}) in all depth zones. The dashed horizontal line indicate a background respiration of zero (conceptually no ER independent of GPP), and the solid lines show significant relationships in LMMs. The centre row shows the relationship of TP with coupling slope (mg O_2 L^{-1} d^{-1}), with the dashed line at a slope of unity (representing a unit increase in ER for each in GPP). The bottom row shows the correction of mean TP with coupling strength (r^2). White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles represent monomictic, dimictic and polymictic lakes, respectively.

Figure 6. Comparison of mean (±SD) daily whole-lake volume weighted (WLVW) metabolism between depth-integrated and single-sensor estimates of (a) GPP, (b) ER and (c) NEP. The dashed line indicates the 1:1 relationship. White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles represent monomictic, dimictic and polymictic lakes, respectively.